53. A method for elucidating a protein expression profile of a test cell line or group of cells, the method comprising:

randomly introducing into the genome of a cell or group of cells a promoterless polynucleotide construct, the construct comprising in a 5' to 3' orientation:

i) a splice acceptor consensus sequence;

ii) the complementary sequence of a type IIS restriction enzyme recognition sequence;

iii) an oligonucleotide sequence encoding an assayable marker peptide;

iv) a polyadenylation sequence;

wherein said promoterless polynucleotide construct when introduced into an actively expressed genes results in the generation of truncated cellular protein fused at its C-terminal to the marker peptide;

v) identifying those cells expressing said marker peptide fused to said truncated cellular protein;

vi) determining the identity of the truncated proteins to which the marker peptide is fused in each group of sorted cells.

54. The method of claim 53 further comprising sorting cells identified in step v) into monoclonal or polyclonal subgroups based on their different levels of expression of said marker peptide.

55. The method of claim 53, wherein the identity of the protein to which the marker peptide is fused is determined using a method selected from the group consisting of 5' RACE and SAVI.

56. The method of claim 55 wherein SAVI is performed by:

i) isolating mRNA from each subgroup of cells;

ii) reverse transcribing the mRNA into double stranded cDNA;

iii) subjecting the cDNA to a restriction enzyme that recognizes the type IIS restriction enzyme recognition sequence, and cleaves the cDNA upstream of the recognition sequence, thereby generating one or more cDNA fragments, wherein each of these fragments comprise the oligonucleotide sequence corresponding to an upstream exon directly fused to the marker peptide, the type IIS

restriction enzyme recognition sequence and a portion of a native sequence corresponding to the peptide marker;

iv) adding an adaptor sequence to the end of the unknown oligonucleotide sequence;

v) amplifying by the polymerase chain reaction, the fragments containing the oligonucleotide sequences of the exons fused to the marker peptide with oligonucleotide primers complementary to the adaptor and peptide marker encoding sequences;

vi) cloning and sequencing said amplified fragments; and

vii) comparing the sequence of each oligonucleotide against oligonucleotide sequences in a one or more nucleotide sequence database thereby identifying one or more fusion proteins present in each subgroup of cells.

57. A method to identify differentially expressed proteins in two different populations of cells, the method comprising:

randomly introducing into the genomes of a reference group of cells and into the genomes of a test group of cells a promoterless polynucleotide construct, wherein the construct comprises, in a 5' to 3' orientation;

i) a splice acceptor consensus sequence;

ii) the complementary sequence of a type IIS restriction enzyme recognition sequence;

iii) an oligonucleotide sequence encoding an assayable marker peptide;

iv) a polyadenylation sequence;

thereby generating a population of randomly truncated cellular proteins fused at their C-terminal truncated end to the marker peptide

v) sorting both groups of cells into several monoclonal or polyclonal subgroups of cells based on their differential expression levels of the marker peptide;

vii) determining the identity of the fusion proteins generated in each subgroup of sorted cells by following one of the following procedures; and

viii) comparing by statistical methods the protein expression profiles obtained for the test group of cells against the protein expression profiles obtained for the reference group of cells, thereby identifying differences in the expression levels of fusion proteins among the two groups of cells.

58. The method of claim 60 wherein the identity of the protein to which the marker peptide is fused is determined by 5' RACE or SAVI.

59. The methods of claims 53-57 or 58 where the peptide marker encoding sequence lacks a translation initiation codon and possesses a translation STOP codon

60. The methods of claims 53-57 or 58 where the peptide marker encoding sequence lacks a translation initiation and STOP codons.

61. The method of claim 56 or 58 wherein addition of the adaptor sequence is performed by ligation of a double stranded adaptor.

62. The method of claim 56 or 58, wherein addition of the adaptor sequence is performed by poly-deoxyribonucleotide tailing extension.

63. The methods of claim 53-57 or 58 wherein said separation of cells into subgroups of cells based on the levels of expression of the peptide marker is performed by fluorescent activated cell sorting.

64. The methods of claims 53-57 or 58 wherein the oligonucleotide sequence is a fluorescent protein coding oligonucleotide sequence.

65. The methods of claim 64, wherein the fluorescent protein encoding oligonucleotide is a green fluorescent protein (GFP) coding sequence.

66. The method of claim 65, wherein the GFP oligonucleotide coding sequence is a humanized rellina GFP (hrGFP) coding sequence.

67. The methods of claims 53-57 or 58 wherein the protein coding sequence is an epitope recognized by fluorescently or enzymatically labeled antibodies.

68. The methods of claims 53-57 or 58 wherein the marker peptide encoded by the polynucleotide requires interaction with another protein in order to generate a fluorescent signal.

69. The methods of claims 53-57 or 58 wherein the polynucleotide construct is introduced into the genome of the cell via a vector.

70. The methods of claim 69, wherein the vector is a viral vector.

71. The methods of claim 70, wherein the viral vector is selected from the group consisting of a retroviral vector, a lentiviral vector, an adenoviral vector, and an adeno-associated viral vector.

72. The methods of claims 53-57 or 58 wherein following amplification of the one or more extended cDNA fragments, and prior to cloning and sequencing the one or more cDNA fragments, the fragments are ligated together to form a concatenated molecule.

73. The methods of claims 53-57 or 58, wherein the polynucleotide construct further comprises, downstream of the oligonucleotide encoding a marker peptide and before the polyadenylation signal, an internal ribosome entry site followed by another protein expression marker.

74. The methods of claims 53-57 or 58 wherein the polynucleotide construct further comprises, downstream of the oligonucleotide having a specified sequence, a sequence encoding, upon expression, a selectable marker.

PRELIMINARY REMARKS

Claims 1-52 have been cancelled and claims 53-74 have been added taking into account the Examiner's objections and the original claims. The claims are generally directed to methods for identifying transcriptionally active regions of the genome of a cell comprising introducing into the genome of a cell a polynucleotide construct, wherein the construct comprises, in a 5' to 3' orientation, a splice acceptor sequence, a sequence complementary, a type IIS restriction enzyme recognition sequence, and an oligonucleotide having a specified sequence which encodes a marker peptide; identifying cells that express the marker as a fusion with a native cellular protein or fragment of a cellular protein isolating mRNA from the cells that express the marker peptide; reverse transcribing the mRNA into cDNA; subjecting the cDNA to a restriction enzyme that recognizes the restriction enzyme recognition sequence, and cleaves the cDNA upstream of the recognition sequence, thereby generating one or more cDNA fragments, wherein each of the one or more cDNA fragments comprise the oligonucleotide encoding the marker peptide, the restriction enzyme recognition sequence, the splice acceptor sequence, and a portion of the